CONTRIBUTIONS TO THE KNOWLEDGE OF THERMOPHILIC ACTINOMYCETES OCCURRING IN CHAMPIGNON COMPOST

by

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It is only from the period of the last 30 years that we have information about comprehensive and detailed examinations of the thermophilic actinomycetes. Haines (1932) has grouped the common saprophytic actinomycetes according to their growth temperature. Knorr (1933) has found such soil sample in which, when exposed to a temperature of 180—200° C, not all of the actinomycetes were destroyed. Jagnow (1957) has examined the effect of temperature and humidity in the proportions of spores and mycelia of various actinomycetes. Thermophilic actinomycetes have been isolated by Gilbert (1904) from soils, by Miehe (1907) from the decomposing masses of plant and by Schütze (1908) from decomposing clover hay. Waksman, Cordon and Hulpoi (1939) have examined in detail the outstanding role played by thermophilic actinomycetes in decomposition processes taking place in thermophilic composts. Henssen (1957) has recognised that certain species of *Streptomyces* and *Nocardia* are found abundantly in thermophilic composts and systematized them mainly according to their morphological properties. The chapter on thermophilic actinomycetes in Waksman's (1961) actinomycetes system includes also the species isolated and identified by Henssen. The systems of Krassilnikov (1949) and Bergey (1957) in respect of actinomycetes, are also important in the investigation of thermophilic actinomycetes.

Krasilnikov and Agre (1965) have described a new species by the name of *Actinobifida chromogena*. Kosmatchev (1959) argued that the thermophilic properties are not sufficient to establish independent genera, and recommended that they should be classified in the same genera as the mesophilic forms.

Blondeau (1959) has demonstrated that the presence of thermophilic actinomycetes acts favourably on the development of the champignon. In the present paper this interaction will be analyzed and some preliminary results will be reported.

Material and Method

1. *Description of the test material*

Compost made of horse manure and coming from the Kőbánya establishment of the Mushroom Growing Enterprise was used as test material. The preparation of compost includes three main phases:

a) the preparative phase (moistening, spreading, etc.)

b) the phase of controlled fermentation

c) the phase of heat treatment.

Due to the activity of microorganisms the temperature of the compost gradually rises during fermentation; after the 12th – 13th day, when temperature is as high as 40 – 45° C, the powdery colonies characteristic of actinomycetes appear in great masses.

It is important that the water content of the compost should not be less than 60 – 65% by the end of fermentation, since this is the optimum level of humidity for microorganisms.

In the course of heat treatment the compost is treated with direct steam of 50 – 60° C in order to destroy the harmful microorganisms. This „pasteurization" takes four days.

The samples examined were taken from various phases of compost preparation:

fresh manure before compost-making (marked I.); fermented compost before heat treatment (II.); compost treated for 24, 48, 72, 96 hours (III – VI.); compost prior to champignon inoculation (VII.); compost after champignon plucking (VIII.).

2. *Isolation of actinomycetes strains and counting of germs*

A 1:10 suspension of compost samples and hot water was made with the shaking technique. Due to the action of hot water the microorganisms attached to the plant parts are more easily detached (B l o n d e a u 1959).

A dilution series was made of this suspension, and 0,5 ml aliquots of the various concentrations were plated on different culture media in Petri dishes. The inoculated culture media were incubated in the thermostat for 3 – 6 days. By means of further plating the pure culture of the strains was obtained.

The following culture media were employed for isolation (W a k s m a n 1961):

a) glycerol-glycine agar (P l o t h o)

b) glucose-asparagine agar (asparagine agar)

c) peptone-beef extract or nutrient agar (pepton agar)

d) sucrose-nitrate agar + 0,6 g yeast extract (Czapek-yeast agar)

Supplemented with actidion, penicillin and nystatin, the same culture media were used for counting the germs of actinomycetes (W i l l i a m s – D a n e s, 1965).

3. *Test method of cellulose decomposition*

The pure actinomycetes strains isolated were examined in two different ways for their capacity of decomposing cellulose:

a) in V i n o g r a d s k y' s standard silica gel solution, with filter-paper in Petri dishes,

b) in deep culture in 50ml flasks, in a culture medium containing only inorganic substances and a cellulose preparation (H u n g a t e 1950) as carbon source. As a control glucose was used as carbon source.

4. *Systematical (morphological) test method*

The microscopic (morphological) examination of active cellulose decomposing strains was made with the hanging-drop method. A culture medium with liquid starch and glucose-yeast was used for preparing a hanging-drop culture. In order to prevent the cultures from running dry, sterile water was poured into the Petri dish under the microscopic slides.

Results and Conclusions

The samples were examined on four different selective culture media, with six series for each sample, under identical conditions. The average numbers of germs after an incubation period of 144 hours, with the various samples and culture media are listed in Table 1.

Table 1.

Average number of germs of thermophilic actinomycetes in I–VIII. compost samples, on four culture media

Samples	Nutrient media			
	Pepton	Czapek	Asp.	Plotho
I	$4 \cdot 10^3$	$2 \cdot 10^3$	$3 \cdot 10^2$	$3 \cdot 10^2$
II	$2 \cdot 10^4$	$3 \cdot 10^4$	$5 \cdot 10^3$	$3 \cdot 10^3$
III	$9 \cdot 10^3$	$7 \cdot 10^3$	$6 \cdot 10^3$	$4 \cdot 10^3$
IV	$4 \cdot 10^3$	$5 \cdot 10^3$	$5 \cdot 10^3$	$4 \cdot 10^2$
V	$2 \cdot 10^3$	$3 \cdot 10^3$	$2 \cdot 10^2$	$3 \cdot 10^2$
VI	$3 \cdot 10^3$	$6 \cdot 10^3$	$3 \cdot 10^2$	$3 \cdot 10^2$
VII	$2 \cdot 10^4$	$3 \cdot 10^4$	$2 \cdot 10^3$	$3 \cdot 10^3$
VIII	$3 \cdot 10^3$	$3 \cdot 10^3$	$7 \cdot 10^2$	$3 \cdot 10$

From the results obtained the following conclusions can be drawn:

1. On all of the four culture media there is a difference in the order of magnitude of the germ number obtained with fresh manure (I) and with compost gained by fermentation (II), respectively. This can be explained by the fact that temperature is rising during compost making as a consequence of the decomposing processes, and this has a beneficial effect on the multiplication of thermophilic microorganisms.

2. In the samples from the heat treatment phase (III – VI.), the germ number decreases on all of the four culture media. This may be due to the inhibited development of thermophilic actinomycetes by radical heat treatment.

3. In the period after heat treatment and prior to inoculation with champignon spores, the compost is undisturbed. As shown by the results of sample VII., this is probably advantageous for the development of actinomycetes.

4. The vigorous growth of the champignon as well as the changed temperature conditions may account for the further decrease of germ number by an order of magnitude, as observed in the sample taken after champignon plucking (VIII.).

5. As shown by the experimental results, thermophilic actinomycetes are growing best on pepton agar and Czapek-yeast agar, whereas their development is less vigorous on the two other media.

In the second part of experiments the pure strains of thermophilic actinomycetes were examined from the point of view of cellulose decomposition. The silica gel method was applied as a qualitative test. A distinction between the activity of the different strains in the liquid culture was made on the basis of growth rate. Combining the results of the two methods, the strains could be grouped on the basis of their cellulose-decomposing capacity as follows:

a) no development	36 strains
b) poor development	18 strains
c) medium development	13 strains
d) vigorous development	6 strains

The quantitative examination of the decomposing capacity of strains developing at a medium and a vigorous rate, respectively, on cellulose media is part of my future work.

On the basis of microscopical examinations and with the help of Henssen's system (Henssen, 1957) the strains of vigorously decomposing capacity could be identified according to their microscopical morphological properties.

Microscopical characteristics of strain TA_{96} *in the hanging drop:*

The vegetative mycelia are non septate, they are branched (Fig. 1.) and twisted in an old culture (Fig. 2.), the aerial hyphae are branched (Fig. 3.), the spores are born one by one on simple and sometimes branched sporophores (Fig. 4.), the spores are round (Fig. 5.) and 1,6 μ in size. All that proves that the said strain belongs to the species *Thermomonospora curvata* Henssen 1957.

Microscopical characteristics of strain TA_{34}*:*

The vegetative mycelia are mainly straight, non-septate (Fig. 6.), aerial mycelia are $0{,}8-1{,}2\,\mu$ thick, $40-50\,\mu$ long and branched (Fig. 7.), the sporophores are spirally curved and formed one by one or in groups from side branches of the aerial mycelia, the round spores are produced in chains of $5-10$, which remain together (Figs. 8., 9., 10.). The data show that this strain is *Thermopolyspora polyspora* Henssen 1957.

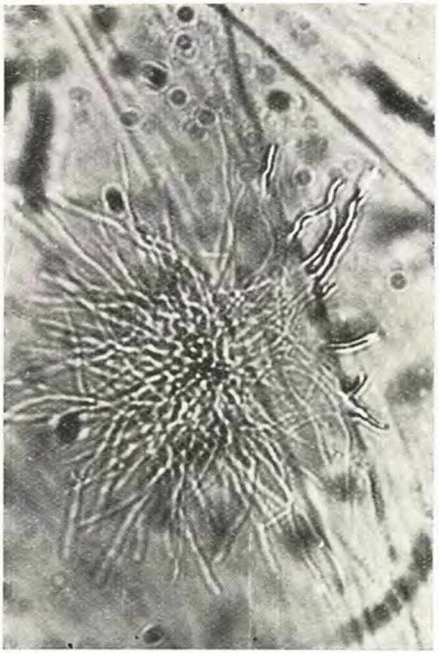

Fig. 1. Vegetative mycelia of *Thermomonospora curvata*. M = 100 : 1

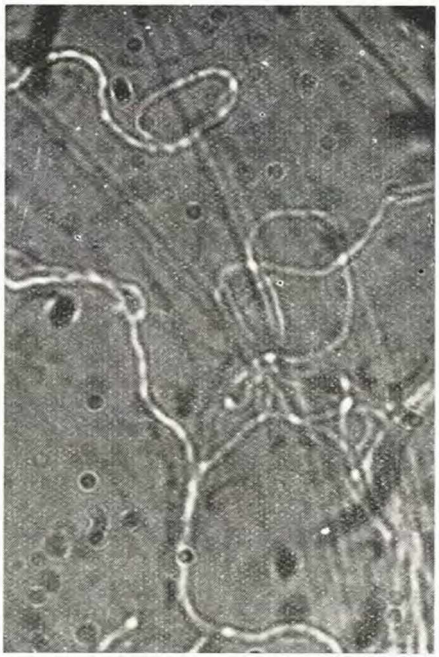

Fig. 2. Vegetative mycelia in an old culture of *Thermomonospora curvata*. M = 272 : 1

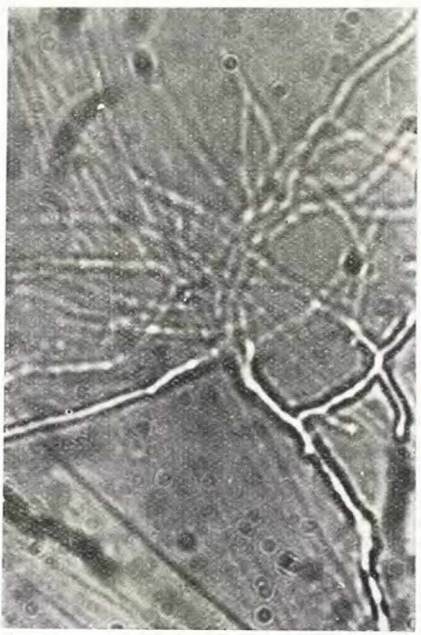

Fig. 3. Aerial mycelia of *Thermomonospora curvata*. M = 272 : 1

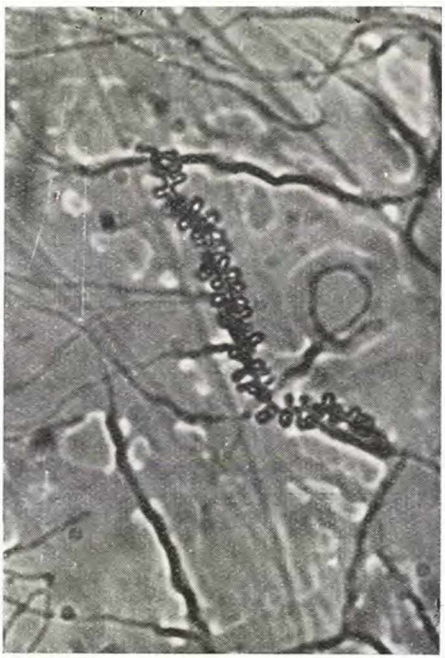

Fig. 4. Sporophoric form of *Thermomonospora curvata*. M = 200 : 1

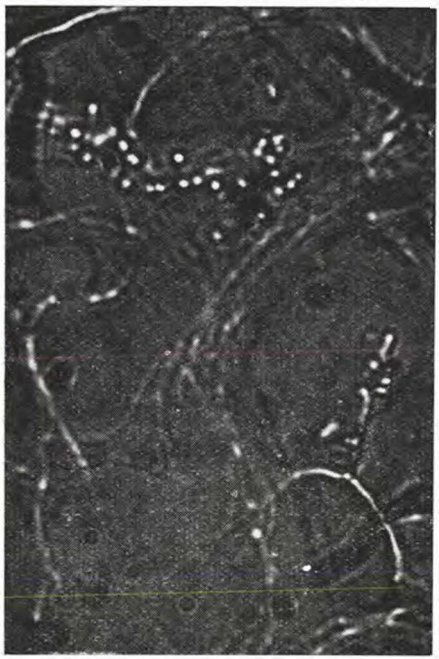

Fig. 5. Spores of *Thermomonospora curvata*. M = 200 : 1

Fig. 6. Vegetative mycelia of *Thermopolyspora polyspora*. M = 200 : 1

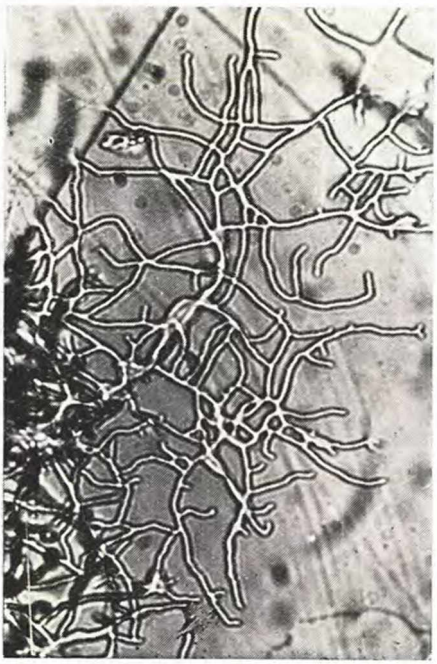

Fig. 7. Aerial mycelia of *Thermopolyspora polyspora*. M = 200 : 1

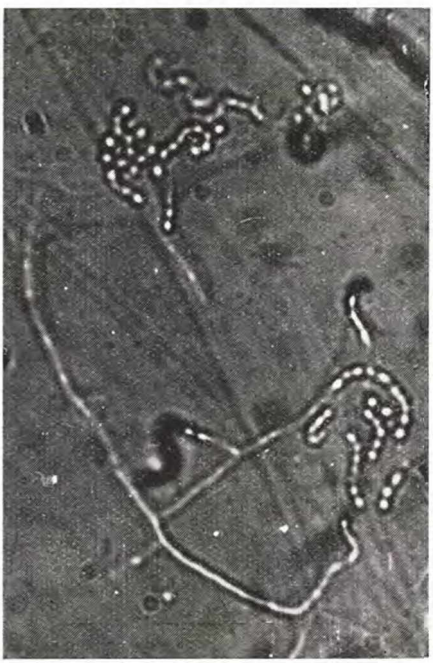

Fig. 8. Sporophoric form of *Thermopolyspora polyspora*. M = 200 : 1

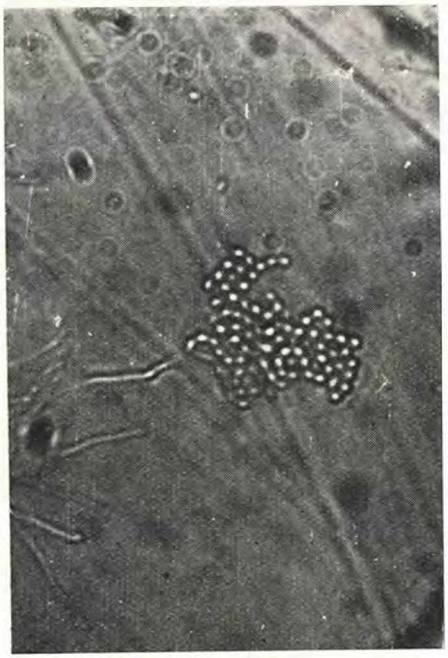

Fig. 9. Spore chains of *Thermopolyspora polyspora* ramaining together, M = 200 : 1

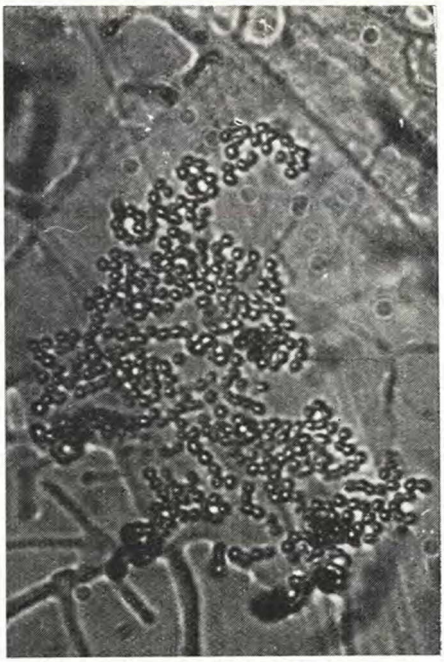

Fig. 10. Spores of *Thermopolyspora polyspora*. M = 200 : 1

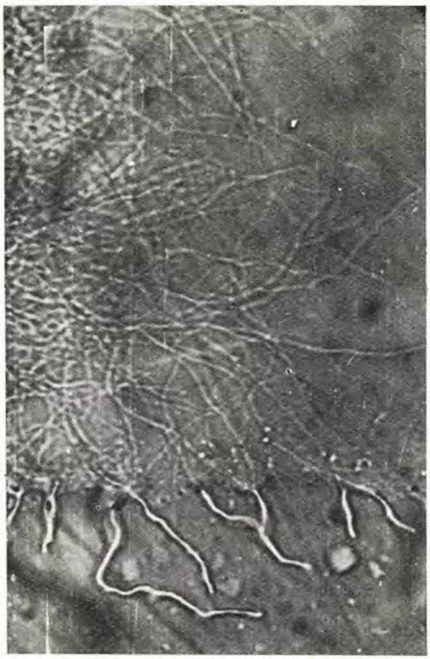

Fig. 11. Vegetative mycelia of *Streptomyces thermovulgaris*. M = 100 : 1

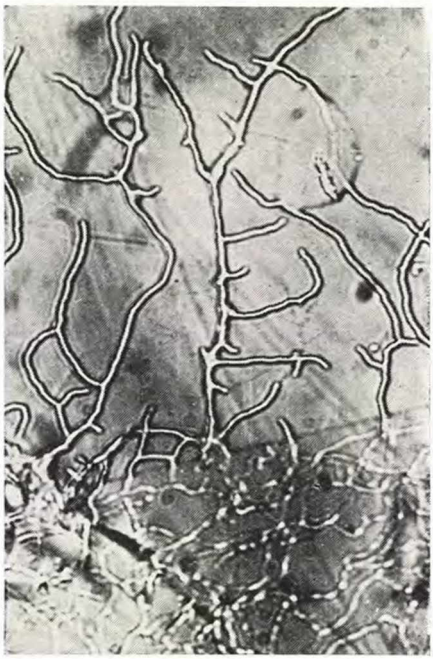

Fig. 12. Aerial mycelia of *Streptomyces thermovulgaris*. M = 100 : 1

Microscopical characteristics of the strains $TA_{40, 42, 50, 58}$:

The vegetative mycelia are not septate (Fig. 11.), the aerial hyphae are branched (Fig. 12.). Spiral sporophores, $16-24$ μ in length, are produced laterally (Fig. 13.), the spores develop in spirals (Fig. 14.) they are oval-shaped

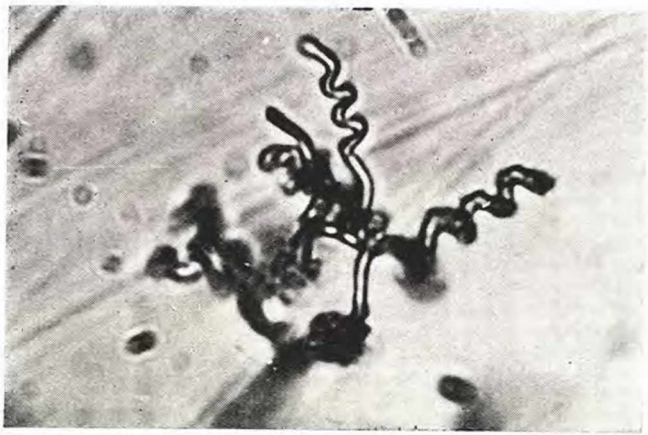

Fig. 13. Spiral sporophores of *Streptomyces thermovulgaris*. M = 200 : 1

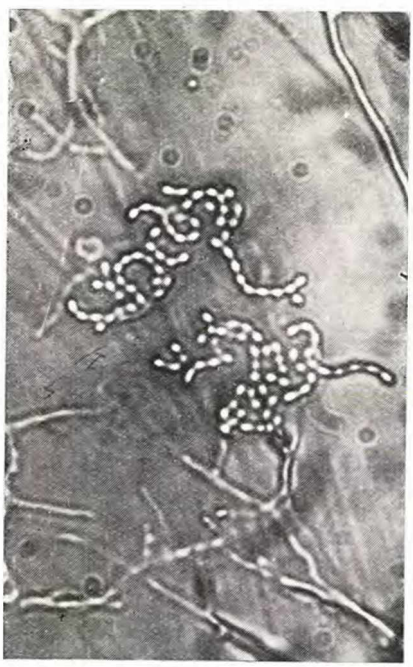

Fig. 14. Spore chains of *Streptomyces thermovulgaris*. M = 200 : 1

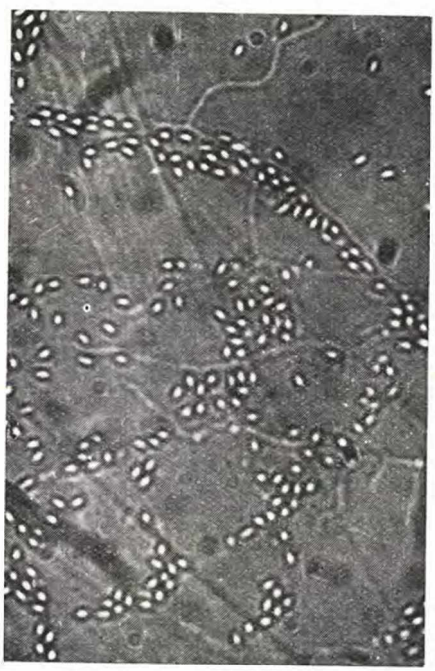

Fig. 15. Spores of *Streptomyces thermovulgaris*. M = 200 : 1

(Fig. 15.) and 0,8 – 1,6 μ in size. These correspond to *Streptomyces thermovulgaris* Henssen 1957.

For their final identification the determination of their physiological properties is needed.

Summary

Experiments were made to establish on four different culture media the number of germs of thermophilic actinomycetes in various stages of compost applied to champignon. Some correlations were found between the development of actinomycetes and that of the champignon. The multiplication of thermophilic actinomycetes was found to be affected by changing temperature conditions during the processes of compost making and heat treatment, as well as in a subsequent phase, by the vigorous growth of champignon. Among the pure strains isolated, 6 proved to be of vigorous activity as far as cellulose decomposition is concerned, and these were identified on the basis of their morphological characteristics.

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